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INFLUENCE OF UPTAKE ROUTE ON THE BIOCONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) BY THE RED SEA BREAM (*Pagrus major*)

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ABSTRACT

Fish species have different responses to polycyclic aromatic hydrocarbons (PAHs) exposure to crude oil. Some achieve PAH biotransformation while others have bioconcentration. These trends may be specie-specific and may depend on PAH uptake route - through water or food. PAH uptake in the red sea bream, *Pagrus major* for PAH biomonitoring in Asian oil spill waters was assessed in water-borne and dietary exposures of the fish to four representative PAHs. In the water-borne exposure, juvenile fish of average weight 83 g were exposed in a flow-through toxicity test for ten days to a mix of 30 µg/L phenanthrene, pyrene, and chrysene; 3 µg/L of benzo[a]pyrene (B[a]P). In the dietary exposure, 20 juveniles of average weight 80 g were fed diets with 10 ng/g phenanthrene, pyrene and chrysene, and 2 ng/g of B[a]P for ten days. Fish livers were sampled for PAH concentrations on days 0, 2, 5 and 10 in both exposures. Three PAHs were accumulated in the water-borne exposure with concentrations increasing with duration of exposure. Phenanthrene had the highest concentration of 2210 ng/g on day 10, while pyrene and chrysene were 170 and 45 ng/g respectively. B[a]P was not accumulated. In the dietary exposure, all the test PAHs were accumulated. Bioconcentration decreased with duration of exposure, unlike in the water-borne exposure which was the reverse. Phenanthrene, with an initial 58 ng/g concentration, was reduced to 10.6 ng/g on day 10. B[a]P accumulation in the dietary exposure is significant. PAH accumulation was higher in the water-borne exposure than in the dietary exposure and PAH bioconcentration in the fish depends on uptake route.

INTRODUCTION

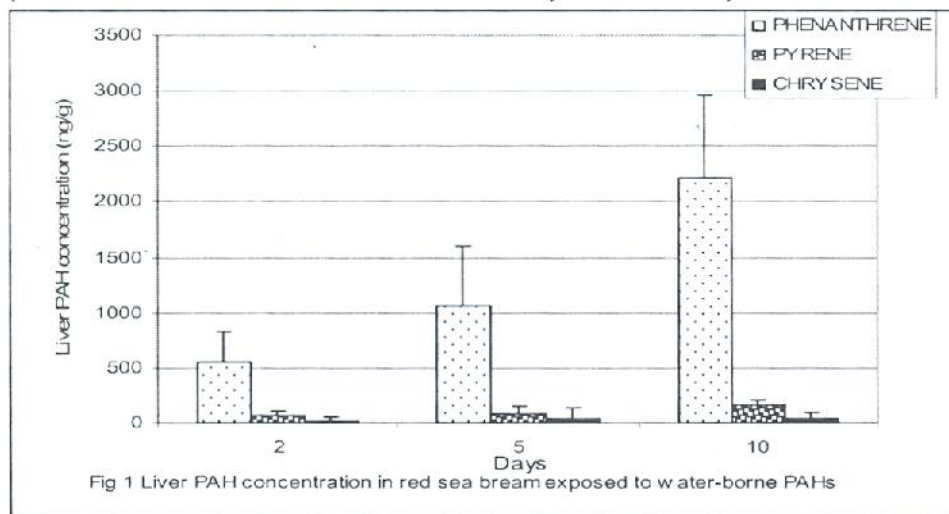
Uptake of PAH in fish is through diffusion from water across the gills, or through the gut from food or PAH-adsorbed sediments (Kelly *et al.*, 2004). The different PAH partitioning between environmental matrices (fish tissue, water and sediments), and species-specific differences in PAH metabolism among fish species and the uptake route may influence PAH accumulation profiles in fish. There are two main routes of uptake of chemicals by aquatic organisms - the water-borne route and the dietary route. Van Veld *et al.* (1997) reported higher hepatic CYP1A induction in the mummichog, *Fundulus heteroclitus*, in water-borne exposures to benzo[a]pyrene (B[a]P) than in diet-borne exposures. Many other species have yielded similar results. The red sea bream, *Pagrus major*, was therefore exposed in this study to water-borne and dietary PAHs to determine their uptake in the liver. Fishes have different habits and habitats with varied food sources. Due to these differences, some fish species may be more susceptible to PAHs bioconcentration in certain compartments of the aquatic environment. Water-borne PAHs may presents challenges that may make them unavailable for bioconcentration. The dietary PAH uptake route has always been disregarded (Randall *et al.*, 1998), but in some species, it may represent a significant source of PAHs to the body (Liang *et al.*, 2007). This study was done to investigate the influence of PAH uptake route on bioconcentration in the red bream.

MATERIALS AND METHOD

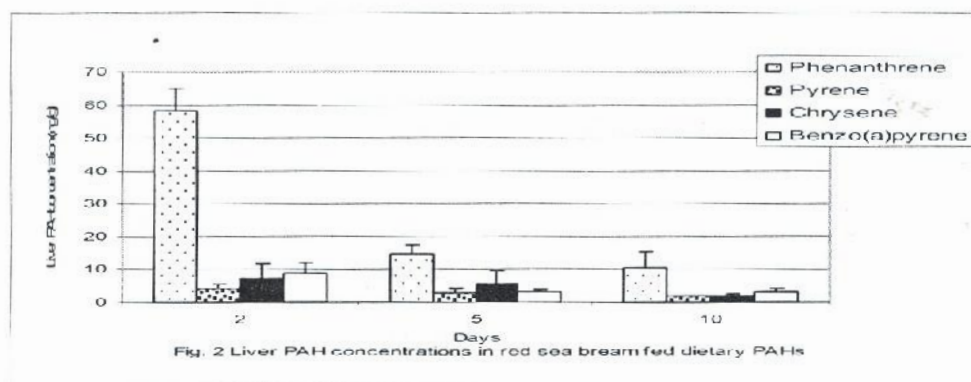
Phenanthrene, pyrene, chrysene, and B[a]P were selected for this study as representative of the 16 PAHs on the United States Environmental Protection Agency priority pollutant list (U.S. EPA, 2007). They range from low molecular weight (LMW) to high molecular weight (HMW) PAHs and represent the whole spectrum of the different physical and chemical properties exhibited by the 16 PAHs. The test fish was separately exposed to water-borne mixtures of the four PAHs in flow-through toxicity experiments; and fed test diets mixed with the four PAHs for 10 days, to compare liver PAH bioconcentration. In the water-borne exposures, PAH concentrations were 30 µg/L phenanthrene, pyrene, and chrysene each, and 3 µg/L B[a]P. The test diets were prepared to contain target concentrations of 10 ng/g each of phenanthrene, pyrene, and chrysene, and 2 ng/g B[a]P. Red sea bream of average weight 84 g were exposed to water-borne PAHs with replicates and a control. In the dietary exposure, 36 g average weights were placed in 200 ml of seawater in replicates in flow-through systems and a control. Fish in the water-borne exposure were not fed during the exposure, but fish in the dietary borne exposure were fed their respective test diets at 3% of body weight per day for 10 days. Water quality parameters of temperature, salinity, dissolved oxygen, and pH, were determined daily in all exposures throughout the duration of the experiment in both exposures. Samples of six fish were removed from the exposure tanks of both exposures on days 2, 5 and 10. The control fish were sampled on days 0 and 10. The fish were dissected, and livers removed, weighed and kept at -20°C for later hepatic tissue PAH concentration determinations. The livers were pooled into three replicates of two fish each for PAH extraction.

RESULTS AND DISCUSSIONS

The liver PAH concentrations in the red sea bream of the water-borne exposures are presented in Fig. 1 below. The control fish livers had PAHs in trace, unquantifiable amounts so they were omitted from results. All accumulation increased with exposure duration to give the highest concentrations of 2210 ng/g phenanthrene, 171 ng/g pyrene, and 46 ng/g chrysene on day 10, while B[a]P was not accumulated at all. PAH accumulation will not occur in species with efficient PAH biotransformation as they are immediately biotransformed on uptake.



More concentrations of LMW PAHs have been reported than the HMW PAHs. The LMW phenanthrene is accumulated in most of these studies. Vives *et al.* (2004) reported a 60% dominance of phenanthrene in trout livers, and Uno *et al.* (2001) reported a similar trend in the livers of the English sole (*Pleuronectes vetulus*). Some fishes do not, however, accumulate B[a]P either due to a lack of B[a]P assimilation or enhanced biotransformation efficiency (Thomann and Kumlos, 1999). In a survey of the Mai Po marshes in Hong Kong, the tilapia (*Sarotherodon mossambicus*) did not accumulate B[a]P even though it was available in the environment, but it accumulated the LMW fluorene, phenanthrene, anthracene, fluoranthene and pyrene (Liang *et al.*, 2007). In a survey of tilapia (*Sarotherodon mossambicus*) of marshes in Hong Kong B[a]P was not detected in the environment, but it accumulated the LMW fluorene, phenanthrene, anthracene, fluoranthene and pyrene (Liang *et al.*, 2007). This study has also confirmed the greater accumulation of the LMW phenanthrene among the accumulated PAHs. Liver PAH concentrations from dietary fed red sea bream are presented in Fig 2 below. All the PAHs were accumulated in the dietary exposure including the HMW B[a]P which was not accumulated in the water-borne exposure. PAH accumulation declined with duration of exposure unlike in the water exposure; phenanthrene was accumulated most with the highest concentration of 58.3 ± 6.9 ng/g and declined to 10.6 ± 4.7 ng/g in day 10. All the PAHs had their lowest concentrations on day 10, unlike in the water-borne exposure where highest concentrations were on day 10. The detection of B[a]P in this red sea bream suggests they will be more readily absorbed in a dietary uptake route. D'Adamo *et al.* (1997) reported an accumulation of B[a]P and 7,12-dimethyl benz(a)anthracene in sea bass fed PAH contaminated mussels. In some species, the water-borne route is a more susceptible route of PAH accumulation, but in others, the dietary route may be more susceptible (Cavret & Feidt, C. (2005). In species with high feeding rates on PAH containing foods or sediments, the dietary uptake route will be critical for PAH accumulation (Liang *et al.*, 2007).



CONCLUSION

This study has shown that the uptake route influences PAH accumulation in the red sea bream. It has shown that the HMW B[a]P can be absorbed in a dietary exposure, but not from a water-borne source.

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